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Biochemical and Biophysical Research Communications 303 (2003) 247–250
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Oxidized LDL receptor gene (OLR1) is associated with the risk of myocardial infarction

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Received 17 February 2003

Abstract

Lectin-like oxidized low-density lipoprotein receptor (LOX-1/OLR1) has been suggested to play a role in the progression of atherogenesis. We analyzed the OLR1 gene and found a single nucleotide polymorphism (SNP), G501C, in patients with ischemic heart disease from a single family, which resulted in the missense mutation of K167N in LOX-1 protein. We compared the group of patients with myocardial infarction (MI) ($n = 102$) with a group of clinically healthy subjects ($n = 102$), and found that the MI group had a significantly high frequency of 501G/C + 501C/C (38.2%) compared with the healthy group (17.6%; $p < 0.002$). The odds ratio for the risk of MI associated with the 501G/C + 501C/C genotype was 2.89 (95% CI, 1.51–5.53). These findings suggest that OLR1 or a neighboring gene linked with G501C SNP is important for the incidence of MI. Manipulating LOX-1 activity might be a useful therapeutic and preventative approach for coronary artery disease, especially for individuals with the G501C genotype of OLR1.

Keywords: Lectin-like oxidized low-density lipoprotein receptor; Single nucleotide polymorphism; Coronary artery disease; Oxidized LDL; Myocardial infarction

Ischemic heart disease, in contrast to rare monogenetic diseases, has been regarded as a complex disease affected by environmental and genetic factors which lead to atherosclerosis of the coronary arteries and the subsequent manifestation of clinical disease [1–5]. Among them, oxidized LDL (Ox-LDL) has been attracting the attention of researchers since it may be the missing link between hypercholesterolemia and atherosclerosis and coronary artery disease [6]. Actually, plasma levels of Ox-LDL were reported to be significantly elevated in patients with acute myocardial infarction compared with patients with stable angina pectoris or healthy controls [7]. Furthermore, Ox-LDL exhibits various biological activities that are crucial for atherogenesis. Ox-LDL transforms macrophages to foam cells, which constitute atherosclerotic plaque. It has been reported that Ox-LDL induces endothelial dysfunction, thus impairing the production of nitric oxide and inducing proatherogenic genes, endothelial–leukocyte adhesion molecules, and smooth muscle growth factors. These activities of Ox-LDL are mediated by a specific but structurally diverse class of receptors, the so-called scavenger receptors.

Lectin-like oxidized LDL receptor-1 (LOX-1/OLR1) was initially identified from vascular endothelial cells as a cell-surface endocytosis receptor for Ox-LDL [8]. The expression of LOX-1 is upregulated by various proatherogenic stimuli, including angiotensin II, TNF-α,

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0006-291X/03/$ - see front matter
doi:10.1016/S0006-291X(03)00326-7

PMA, and lysophosphatidylcholine. In endothelial cells, ligand binding to LOX-1 induces the production of leukocyte adhesion molecules, chemokines, endothelin-1, and the superoxide anion, and decreases the release of nitric oxide, which are relevant to endothelial dysfunction. These features of LOX-1 suggest that it might play a proatherogenic role and promote the occurrence of coronary artery disease. Here, we examined the significance of LOX-1 in coronary artery disease from the aspect of genetics, identified a single nucleotide polymorphism (SNP) in the human OLR1 gene, and revealed the association of the SNP with myocardial infarction (MI) for the first time.

Materials and methods

Subjects. Peripheral blood samples were collected from patients with MI, their family members, and controls. One hundred and two patients with myocardial infarction (defined as a history of chest pain associated with elevation of regional ST segment elevation and serum creatine kinase) were recruited between 1971 and 1996 at the Heart Institute of Japan, Tokyo Women's Medical University. Age- and sex-matched controls without hyperlipidemia were recruited from the same geographical area as the patients. Individual informed medical consent was obtained from all subjects.

Lipid analysis. Serum lipids were determined from fresh samples. Total cholesterol and oxidized low-density-lipid were measured by Kyowa Medics (Tokyo, Japan).

Genetic analysis. RNA and DNA were prepared according to a method reported previously [9].

Reverse transcriptase-polymerase chain reaction for OLR1 mRNA expression. Total RNA (1 μg) extracted from peripheral blood lymphocytes was reverse transcribed with an outer reverse primer (antisense primer, 5′-gtttttctgggctctcatgtttggcaccc-3′) with Super Script II (Gibco-BRL, Life Technologies, Tokyo) at 45 °C for 90 min. The reverse-transcribed material (10 μl) was amplified with *Taq* DNA polymerase (Perkin–Elmer) using an outer forward primer specific to human OLR1 gene (sense primer, 5′-tcgtgactgcttcactctctcattcttagc-3′). The first PCR product (1 μl) was amplified using an inner primer pair of human OLR1 (sense primer, 5′-ctctcattcttagcttgaatttgg-3′, antisense primer, 5′-tttggcacccaagtgacaaagaat-3′). The second PCR product was 738 bp. The temperature of the first PCR was 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min and that of the second PCR was 90 °C for 45 s, 55 °C for 90 s, and 72 °C for 2 min. Both reactions were amplified for 35 cycles. The RT-PCR-amplified samples were visualized on 2% agarose gels using ethidium bromide.

OLR1 sequence. The dideoxynucleotide chain method [10] was used to sequence the cDNA insert using modified T7 DNA polymerase (Sequenase Version 2.0, United States Biochemical, Cleveland, OH) to incorporate fluorescein-dATP (Fluore-dATP Labeling Mix, Pharmacia, Uppsala, Sweden) and it was analyzed with a A.L.F. DNA sequencer (Pharmacia). Nucleotide and deduced amino-acid sequences were analyzed using Genetics Gene Analysis System Software (Genetics, Tokyo/Japan).

Single-strand conformation polymorphism analysis. The sequences of primers used in the polymerase chain reaction (PCR) were 5′-ttgcttg ctggatgaagtcc-3′ and 5′-attgttcagctccttgtccg-3′. Samples of 100 ng genomic DNA template and 50 pM of each primer were used in the PCR. Denaturation was at 94 °C for 10 s and annealing at 55 °C for 20 s, with extension at 72 °C for 20 s with a 30-cycle amplification. PCR was performed in a buffer containing 1.5 mM $MgCl_2$, 0.8 mM dNTP. For the SSCP analysis, amplified fragments were electrophoresed on commercialized polyacrylamide gels (GeneGel Excel Kit, Pharmacia Biotech, San Francisco, CA) at 16 °C, at 600 V for 60 min. The gels were visualized by a silver-staining protocol using Hoefer Automated Gel Stainer (Pharmacia Biotech, San Francisco, CA). In the SSCP electrophoresis, the single-strand product with the mutation, which was analyzed by direct sequencing, demonstrated different band motilities on the gel.

Statistical analysis. The distributions of the single nucleotide polymorphism (SNP) and allele frequencies for patients and control subjects were compared using the χ^2 test. The distributions of genotype in the patients and control subjects were in Hardy–Weinberg equilibrium. Statistical significance was taken to be $p < 0.05$. The odds ratio (OR) was calculated as estimators of relative risk, together with their 95% approximate confidence intervals (95%; CI). Association with conventional risk factors was tested by analysis of variance adjusted for age and gender.

Results

To investigate the potential role of LOX-1 in human ischemic heart disease, we first searched SNPs of OLR1 in a family with MI patients, in which five out of thirteen family members suffered from ischemic heart disease. We analyzed cDNA from peripheral blood lymphocytes of the family. An SNP (GenBank Accession No. AB102861), G-to-C at position 501, which results in a missense mutation of Lys to Asn at position 167 (K167N), was found in four patients with coronary heart disease (CHD) (II-1, 3, 4, and 5) and in a no CHD but still young (32 years old) family member (III-2)

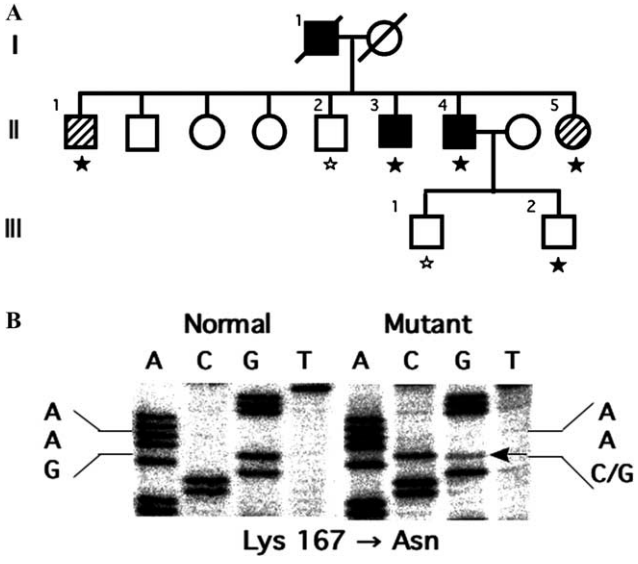

Fig. 1. (A) Pedigree, clinical features, and mutations of OLR1 in a family with myocardial infarction. Patients with myocardial infarction are indicated by filled squares (males). Patients with angina pectoris are indicated by oblique lined circles (female) or squares. The K167N mutation carriers are indicated by filled asterisks. Subjects without the mutation are indicated by empty asterisks. (B) DNA from a patient (right) and a normal individual (left) was analyzed. The DNA sequence shows a mutation of a G-to-C substitution, which results in the substitution of lysine by asparagine in the OLR1. This mutation was heteroplasmic in subjects II-1, 3, 4, 5, and III-2.

Table 1
Patient basic demographics and lipid levels

ID	II-1	II-2	II-3	II-4	II-5	III-2
Age (years)	77	67	65	63	60	32
Gender	Male	Male	Male	Male	Female	Male
TC (mg/dl)	217.7	286.5	298.6	246.2	262.4	225.1
Ox-LDL (U/ml)[a]	29.1	21.5	16.3	20.5	30.7	43.0
Anti-lipid drugs	–	–	+	+	+	+
CHD	+	–	+	+	+	–
CABG	–	–	+	+	–	–

TC, total cholesterol; Ox-LDL, oxidized low-density lipoprotein; CHD, coronary heart disease; CABG, coronary artery bypass grafting.
[a] The mean level of serum Ox-LDL is 11–13 U/ml.

(Fig. 1). Numbering for the SNP starts at the adenine nucleotide (A) in the ATG initiation codon in the cDNA (GenBank NM-002543.1). Patients I-1, II-3, and II-4 had a history of MI, and patients II-1 and II-5 had a history of angina pectoris. All the members of this family showed higher serum levels of total cholesterol and Ox-LDL, although patients II-3, 4, and 5 and subject III-2 had been medicated with HMG-CoA reductase inhibitor (Table 1).

To evaluate the association of the K167N mutation and the incidence of MI, SSCP analysis was performed with genomic DNA specimens from 102 patients with MI (mean 55.3 years, 84 males and 18 females) and age- and sex-matched control subjects (mean 55.3 years, 84 males and 18 females). The genotype distributions were in Hardy–Weinberg equilibrium and are given in Table 2. The population with K167N mutation of OLR1 (501G/C + 501C/C genotype) was significantly larger in the MI group (38.2%) than in the control group (17.6%). The difference was statistically significant ($p < 0.002$). The odds ratio for risk of MI associated with the 501G/C + 501C/C genotype was 2.89 (95% CI, 1.51–5.53). The frequency of the 501C allele was found to be significantly higher in the MI group (20.6%), compared with the control group (9.3%; $p < 0.002$) (Table 3).

Table 2
Distribution of the OLR1 G501C genotypes in cases and controls

	Cases of myocardinal infarction ($n = 102$)	Controls ($n = 102$)	p
501G/G	63 (61.8%)	84 (82.4%)	
501G/C + 501C/C	39 (38.2%)	18 (17.6%)	<0.002

p was calculated using the χ^2 test.

Discussion

Accumulating evidence suggests that LOX-1 might be involved in the induction of endothelial dysfunction caused by Ox-LDL, which may lead to the initiation and progression of atherosclerosis. In the present study, we identified a SNP, G501C, in the open reading frame of the OLR1 gene from a family with a high incidence of MI. Four out of five of the brothers investigated were suffering from ischemic heart diseases, and had the 501G/C genotype, while the other healthy brother had the 501C/C genotype. Furthermore, compared with sex- and age-matched controls, we found a significantly higher population with the 501G/C or 501C/C genotype in the MI group. The significant association of the 501C genotype of OLR1 with a risk of coronary artery disease may further support the importance of LOX-1 in the pathogenesis of atherosclerosis.

The G-to-C change results in the missense mutation of K167N. The amino-acid residue 167 is located at the C-type lectin-like domain in the extracellular portion of LOX-1. A previous study demonstrated that the lectin-like domain recognizes ligands and the basic amino-acid residues are important for strengthening ligand-binding [11,12]. Although we did not examine the effects of K167N in the study, the K167N mutation that changes basic amino acid residue to neutral amino acid may attenuate the ligand–receptor interaction. However, recent studies have suggested that LOX-1 is a proatherogenic molecule, which induces expression of leukocyte adhesion molecules, a chemoattractant molecule, MCP-1, AT1 receptor, and endothelin-1, increases the generation of reactive oxygen species, and decreases NO synthesis in endothelial cells [13]. Considering these lines of evidence, we are speculating that the G-to-C change

Table 3
Allele frequencies of the OLR1 G501C in cases and controls

Allele	Alleles, n(%)		Total	p
	Cases of myocardial infarction	Controls		
G	162 (79.4)	185 (90.7)	347	
C	42 (20.6)	19 (9.3)	61	<0.002

p was calculated using the χ^2 test.

at 501 or neighboring SNPs in disequilibrium may increase the expression level or binding activity of LOX-1. A functional study on the activity of LOX-1 associated with the 501C genotype would help further the understanding of the role of LOX-1 in atherosclerosis.

Interestingly, Welch et al. [14] mapped two atherosclerosis susceptibility loci in mice by interstrain genetic cross of LDLR knockout mice. One of the loci resides on the region corresponding to human chromosome 12p13-12, where we mapped the OLR1 gene [15]. Although they did not identify the gene carrying atherosclerosis susceptibility, the phenotype of the mice carrying the gene fits the character of LOX-1 well. The susceptibility was independent of plasma total cholesterol, high-density lipoprotein cholesterol, non-high density lipoprotein cholesterol, insulin, and body weight. On the other hand, LOX-1 is a receptor for oxidatively modified lipoprotein and closely related to oxidative stress, which induces LOX-1 expression and is induced by LOX-1 [16,17]. As oxidized LDL has been proposed as the factor that affects atherogenesis beyond cholesterol metabolism [6], it is likely that the receptor closely related to oxidative stress might work as an atherosclerosis-susceptibility molecule independent of cholesterol. We cannot exclude, however, other gene(s) that may be in linkage-disequilibrium with the 501C SNP from the candidate. Therefore, we must wait for a study in a larger population. However, we would like to suggest that manipulation of LOX-1 activity might be advantageous therapeutically, especially for the patients who carry the 501C allele of OLR1.

Acknowledgments

We are grateful to Ms. B. Levene for reading the manuscript and to Dr. N. Kamatani and Dr. Y. Nakamura, Institute of Rheumatology, Tokyo Women's Medical University, for advice with the statistical analysis. The research was supported by the Grant for the Promotion of the Advancement of Education and Research in Graduate Schools (2000, 2001).

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